Synthesis of Biotin-FlAsH and Eosin-FlAsH Fluorescent Probes

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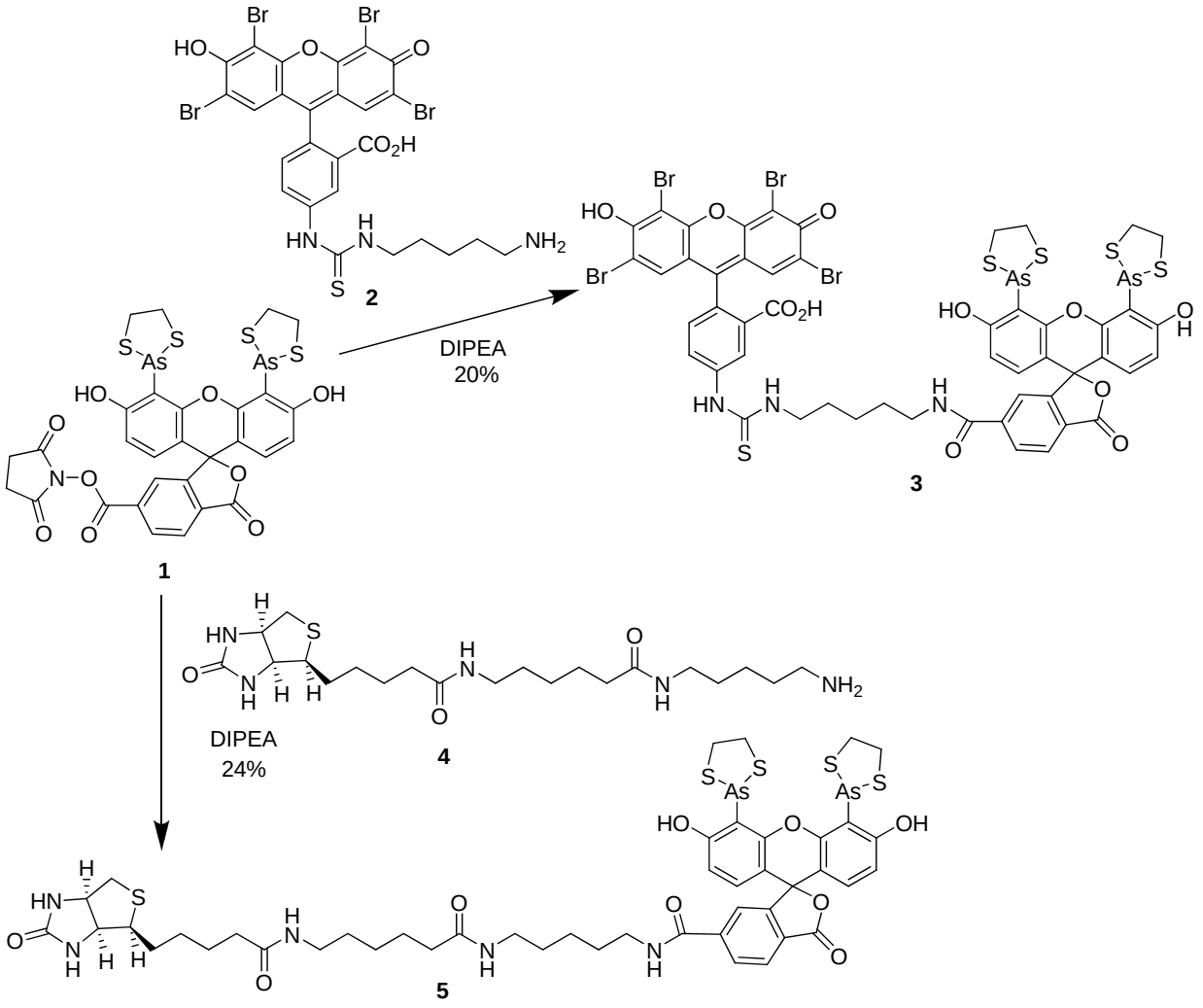
Background

The biarsenical fluorophore FlAsH is useful for the specific labeling of protein tetracysteinyl-(TC) motifs in many protein systems,[1] and may be useful for studies related to mechanisms of prion infection.[2] An understanding of how prion infection occurs and is spread, from the use of detectable molecular probes, may lead to therapeutic methods for inhibition of infection. Detectable agents based on the TC-binding biarsenical FlAsH dye additionally coupled to secondary recognition and detection agents are useful in this regard, and for this reason we made the title conjugates wherein FlAsH is coupled to biotin and eosin, respectively.

Chemistry

The preparation of FlAsH-biotin and -eosin conjugates was based on standard conjugation chemistries, as shown in **Scheme 1**, using the 6-hydroxysuccinimidyl ester of the FlAsH dye. In both reactions diisopropylethylamine (DIPEA) was used as base and a cadaverine-eosin (**2**) and a long-chain derivative of biotin (**4**) were used to generate eosin-FlAsH and biotin-FlAsH, respectively.

Scheme 1. Coupling of N-hydroxysuccinimidyl-FlAsH to amino derivatives of eosin and biotin



Experimental

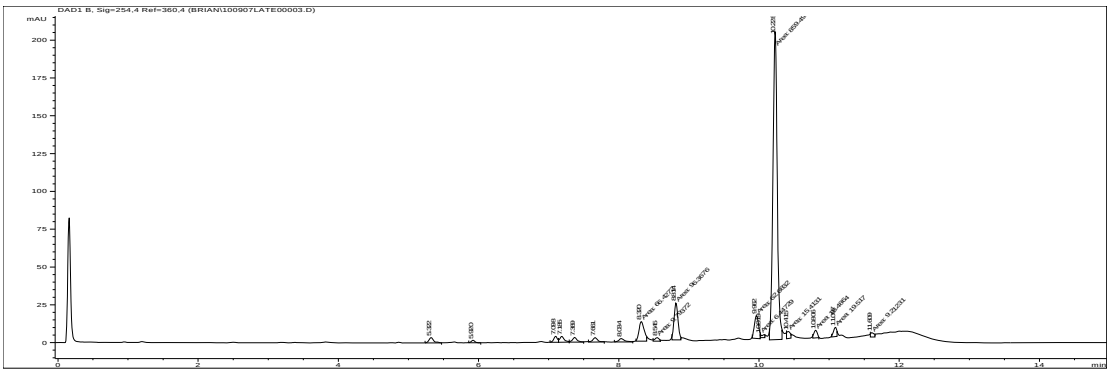
General. Eosin-cadaverine and biotin-cadaverine were purchased from Invitrogen. Analytical Silica gel 60 F254 TLC plates were from Merck and Preparative Silica gel GF 2000μm 20x20cm plates from Analtech. Flash chromatography was done on an Analogix Intelliflash Workstation with a SuperFlash Sepra Si 50 SF column. HPLC analyses were performed using an Agilent 1200 Series High Performance Liquid Chromatography System. Mass spectral analyses were done on an Agilent 1200 mass spectrometer.

Coupling of FlAsH succinimidyl ester to eosin-cadaverine. Compound (**1**) (4.8 mg, 6.0 μmol) was dissolved in DMSO (150 μL). To the solution was added eosin-cadaverine (**2**) (4.8 mg, 6.0

μmol) and DIPEA (2.0 μL, 11 μmol). The resulting solution was shaken in dark for 24 hours at room temperature. The solution was purified by preparative TLC (developed in CH_2Cl_2/MeOH, 5:1) and HPLC to give product (**3**) as an orange solid. Yield, 1.8 mg (20%). Both the preparative HPLC purification and the analysis by analytical HPLC used 0.1% TFA in water as solvent A and 0.1% TFA in water as solvent B. For preparative HPLC purification an Agilent XDB-C_{18}, 5.0 μm, 21.2x50 mm column with a linear gradient of from 5-95% solvent B was used at a flow of 20mL/min, λ = 254 nm. For HPLC analysis an Agilent XDB-C_{18}, 3.5 μm, 2.1x50 mm column was used with the same gradient as the preparative work, but with a flow rate of 1mL/min. The product (**3**) eluted at 10.2 minutes, λ = 254 nm (**Figure 1**).

Coupling of FlAsH succinimidyl ester to biotin-cadaverine. Compound (**1**) (4.8 mg, 6.0 μmol) was dissolved in DMSO (150 μL). To this solution was added biotin-cadaverine (**4**) (3.3 mg, 6.0 μmol) and DIPEA (2.0 μL, 11 μmol). The resulting solution was shaken for 24 hours at room temperature. The solution was purified *via* preparative TLC (developed in CH_2Cl_2/MeOH, 5:1) to give product (**5**) as a pink solid. Yield, 1.6 mg (24%). MS (m/z): 1132.3 $[M+1]^+$. For HPLC analysis an Agilent XDB-C_{18}, 5.0 μm, 4.6x150 mm column was used with a flow rate of 1.0 mL/min. Solvent A was 0.05% TFA in water and solvent B was 0.05% TFA in acetonitrile. A linear gradient from 25-75% acetonitrile was used with (**5**) eluting at 5.0 minutes, λ = 254 nm

(**Figure 1**).



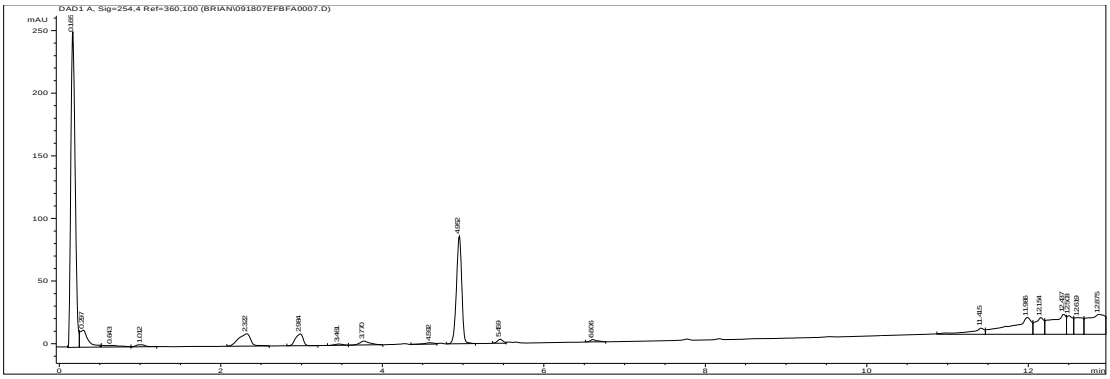


Figure 1. HPLC elution traces of the FlAsH-eosin (top) and FlAsH-biotin (bottom) conjugates measured at an absorbance of 254 nm. X-axis is time (minutes); y-axis is UV absorbance (arbitrary units) of eluting compound.

References

1. Tsien RY. Building and breeding molecules to spy on cells and tumors. FEBS Lett. 2005 Feb 7;579(4): 927-32. PMID 15680976

2. Magalhaes AC, Baron GS, Lee KS, Steele-Mortimer O, Dorward D, Prado MA, Caughey B. Uptake and neuritic transport of scrapie prion protein coincident with infection of neuronal cells. J Neurosci. 2005 May 25;25(21): 5207-16. PMID 15917460